attractions such that the dye groups self-quench their fluorescence by dye dimerization or stacking, and

b) cleaving one or more of said cleavable bonds of the peptide by said characteristic enzyme to release the fluorescence dye groups from dye dimerization or stacking, thereby producing an at least 20-fold increase in fluorescence intensity over that of the quenched dye groups thereby indicating the presence of said microorganism, wherein the emission wavelength of the fluorescent dye groups is at or above 570 nm.

D3

Remarks

Claims 2, 11 and 14 have been canceled.

Pending claims in view thereof are claims 1, 4-10 and 12-21.

Claim 20 is allowable.

The objection to claims 2 and 14 is now moot.

Reconsideration is respectfully requested of the rejection of the pending claims under 35 U.S.C. 103(a) as allegedly unpatentable over Garman et al. (GB 2278356) as supported by Rohatgi et al., and Wei et al., and in view of Komoriya et al. (U.S. 5,714,342).

The rejection of claims 9 and 19 under 35 U.S.C. 103 over the same references above further in view Heath, Jr., is also traversed for the reasons provided below concerning the main rejection. Withdrawal of the main rejection of the main claims render claims 9 and 19 allowable since they will be dependent on allowable claims.

Claim 21 has been rejected under 35 U.S.C. 103(a) as allegedly unpatentable over the same references cited in the rejection of claims 1, 4-8, 10-18 further in view of Manafi et al.

This rejection is also traversed based on the same reasons provided below in rebutting the initial rejection.

Claims 1, 12 and 21 have been amended to further distinguish over the references cited. The invention claimed provides for at least 20-fold increase in fluorescence intensity over that of the quenched dye groups thereby indicating the presence of said enzyme. Support for the 20-fold increase can be found in the specification at page 14, line 27 and particularly in Table 1 where experimental results show in every case intensity ratios of greater than 20-fold.

The Examiner's rejection of the claims appears to be based principally on Garman as the primary reference and Komoriya as essentially the secondary reference. The Examiner has made a statement regarding Garman that it allegedly teaches "substrates comprising two groups with the same fluorophore (substrate 3 on page 11)". This appears to be an error. In reading the passage at page 11, one also has to look at page 10. First of all, the reference substrate 3 should be substrate B since it follows from a description of substrate A and is between a further description on page 11 of substrate C. The three different substrates having different fluorophores are prepared from [Cys-26] fluororesceinyl peptide I described at page 10. It is clear that three different fluorophores are used in coupling to the peptide to prepare substrates A and B and C.

In the outstanding Office Action, the Examiner has admitted:

> Garmen, as supported by Rohatgi and Wei, does not teach "at least" a 10-fold increase in fluorescence intensity and its method compared to "conventional assay kits" using a protease substrate; nor does Garman, as supported by Rohatgi and Wei teach a substrate wherein the dye groups are identical, have emission wavelengths of 570 nm or more, and give at least a 10-fold increase in fluorescent intensity upon separation of the dye groups."

However, the Examiner has combined these references with Komoriya as allegedly providing the missing description in the above three references to allegedly render obvious the claimed invention. This contention is rebuttable based on the amendment to the claims and the data in the present specification at Table 1 showing that the fluorescence intensity is at least 20-fold or greater with identical dyes. In contrast, Komoriya teaches away from the use of identical fluorescent dye groups. In Table 9, columns 32-33 of Komoriya, where the dye group is the same, the fluorescence intensity is not greater than 10-fold. In fact, it is a 10-fold increase or less when calculating I_c/I_o. Greater than 10-fold increases are noted only where the dye groups are different. Thus, it is clear that the present invention as claimed and supported by the data in the specification overcomes the references cited. In view thereof, the Examiner's rejection should be withdrawn.

A prompt and favorable reply is earnestly solicited.

Respectfully submitted,

4/30/03
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Claims 2, 11 and 14 have been canceled.

Claims 1, 12 and 21 have been amended as follows:

1. (Four times amended) A method of biological assay comprising:

a) providing an enzyme substrate comprising two or more identical fluorescence dye groups bound to a flexible peptide comprising one or more enzymatically cleavable bonds, the dye groups being drawn together by free energy attractions such that the dye groups self-quench their fluorescence by dye stacking or dimerization, and

b) contacting said substrate with a substance being assayed to determine the presence of an enzyme capable of cleaving an enzymatically cleavable bond wherein the enzymatic cleaving of said cleavable bond of the peptide will release the fluorescent dye groups from dye stacking or dimerizing, thereby producing an at least [10-fold] 20-fold increase in fluorescence intensity over that of the quenched dye groups thereby indicating the presence of said enzyme, wherein the emission wavelength of the fluorescent dye groups is at or above 570 nm.

12. (Four times amended) A protease substrate comprising a flexible peptide and including two identical fluorescence dye groups that are drawn together by free energy attractions so as to self-quench fluorescence of the dye groups by intramolecular dimerization or stacking and which, when separated, fluoresce at an at least [10-fold] 20-fold increase in fluorescence

intensity over that of the quenched dye groups, wherein the emission wavelength of the fluorescent dye groups is at or above 570 nm.

21. (Four times amended) An assay method of detecting a microorganism, which microorganism produces a characteristic enzyme, comprising:

a) providing an enzyme substrate specific for said characteristic enzyme produced by said microorganism comprising two or more identical fluorescence dye groups bound to a flexible peptide comprising one or more bonds cleavable by said characteristic enzyme, the dye groups being drawn together by free energy attractions such that the dye groups self-quench their fluorescence by dye dimerization or stacking, and

b) cleaving one or more of said cleavable bonds of the peptide by said characteristic enzyme to release the fluorescence dye groups from dye dimerization or stacking, thereby producing an at least [10-fold] <u>20-fold</u> increase in fluorescence intensity over that of the quenched dye groups thereby indicating the presence of said microorganism, wherein the emission wavelength of the fluorescent dye groups is at or above 570 nm.